THERMOPHILIC AND THERMOTOLERANT FUNGI ASSOCIATED WITH SEEDS OF FIVE MEMBERS OF *UMBELLIFERAE* FROM EGYPT

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SUMMARY — Eighteen thermophilic and thermotolerant species in addition to 2 varieties belonging to 13 genera were isolated from seeds of caraway (11 genera and 15 species), cummin (10 genera and 15 species), fennel (9 genera and 12 species), anise (8 genera and 11 species) and coriander (7 genera and 10 species) ▬ glucose-Czapek's agar at 45°C. Total counts of thermophilic and thermotolerant fungi fluctuated according to the samples tested between 24-432, 30-496, 8-1000, 12-572 and 30-520 colonies per ▮ dry seeds in the five types of seeds, respectively. *Aspergillus fumigatus, Emericella nidulans* and *Rhizomucor pusillus* were the most prevalent fungi in all types of seeds. Truly thermophilic fungi were isolated, but with variable densities and frequencies, from the various types of seeds and these were *Chaetomium thermophilum* var. *coprophilum, Humicola hyalothermophila, H. grisea* var. *thermoidea, Malbranchea pulchella* var. *sulfurea, Melanocarpus albomyces, Myceliophtora thermophila, Talaromyces dupontii* and *Thermomyces lanuginosus.*

RÉSUMÉ — Dix-huit espèces et 2 variétés thermophiles et thermotolérantes, appartenant à 13 genres, ont été isolées de graines de carvi (11 genres et 15 espèces), cumin (10 genres et 15 espèces), fenouil (9 genres et 12 espèces), anis (8 genres et 11 espèces) et coriandre (7 genres et 10 espèces) sur milieu Czapek (glucose), à 45° C. Les nombres de champignons thermophiles et thermotolérants fluctuent, respectivement dans les cinq types de graines, entre 24-432, 30-496, 8-1000, 12-572 et 30-520 colonies par gramme de graines sèches. *Aspergillus fumigatus, Emericella nidulans* et *Rhizomucor pusillus* sont les champignons les plus fréquents dans tous les types de graines. Des champignons thermophiles ont été isolés, mais avec des dentités et des fréquences variables, ce sont *Chaetomium thermophilum* var. *coprophilum, Humicola hyalothermophila, H. grisea* var. *thermoidea, Malbranchea pulchella* var. *sulfurea, Melanocarpus albomyces, Myceliophtora thermophila, Talaromyces dupontii* et *Thermomyces lanugisosus.*

KEY WORDS : Seed-borne, thermophilic fungi, thermotolerent fungi, caraway, cummin, fennel, anise, coriander.

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INTRODUCTION

Thermophilic and thermotolerant fungi have been mostly associated with either heat-damaged or stored seeds from different places of the world (MULINGE & APINIS, 1969 ; MULINGE & CHESTERS, 1970 ; MILLS & BOLLEN, 1976 ; FLANNIGAN, 1969). These fungi were also isolated from stacks of oil palm kernels in Nigeria (OSO, 1974) and were found to cause serious deterioration during storage of the palm kernels (OSO, 1979) or palm produce (OGUNDERO, 1981a).

In Egypt, several investigations have been carried out on the frequencies of occurrence and densities of mesophilic seed-borne fungi (MOUBASHER & al., 1972, 1979, 1980, 1983 and others), but only that of MOUBASHER & al. (1979) and ABDEL-HAFEZ & SHOREIT (1986) dealt with thermophilic fungi associated with peanut, bean, lupine, and pea seeds. The present investigation aimed to study the densities and frequencies of occurrence of these fungi associated with seeds of five medicinal plants of the family *Umbelliferae* commonly used and cultivated in Egypt for their nutritive and medicinal values.

MATERIALS AND METHODS

Forty samples of each of caraway (*Carum carvi* L.), cummin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* Miller), anise (*Pimpinella anisum* L.) and coriander (*Coriandrum sativum* L.) seeds of crop 1986, of 250 g each, were collected from the market in eight Governorates from Egypt namely, Cairo, Giza, Beni-suef, El-Minya, Assiut, Sohag, Qena and Aswan. The number of samples from each governorate and seed type was five.

Determination of thermophilic (or thermotolerant) seed-borne fungi :

The dilution-plate method was used as employed by MOUBASHER & al. (1972). Czapek's agar medium was used in which glucose (10 g/l) replaced sucrose. Rose bengal (66 ppm) was added as a bacteriostatic agent (SMITH & DAWSON, 1944). Five plates were used for each sample and were incubated at 45°C for 7-10 days and the developing fungi were counted, identified and calculated per g dry seeds.

RESULTS AND DISCUSSION

Total counts of thermophilic and thermotolerant fungi were considerably high (Table 1) and anise seeds were the richest in total fungal population (8 700 colonies per g seeds in forty samples) followed by cummin (7 370 colonies), fennel (7 320 colonies), coriander (5 340 colonies) and caraway seeds (5 020 colonies). Total counts of these fungi ranged between 12-572, 30-496, 8-1000, 30-520, and 24-432 colonies per g dry seeds, respectively. ABDEL-HAFEZ & SHOREIT (1986) found that total counts of thermophilic and thermotolerant fungi were generally low in pea (193.2 colonies), bean (79.8 colonies) and lupine seeds (46.2 colonies per g in all samples ; 30 samples each) collected from eight Governorates in Upper Egypt.

Thirteen genera and eighteen species in addition to one variety of each of *E. nidulans* and *A. fumigatus* were identified on the whole. Broadest spectra of genera and species was recorded in caraway (11 genera and 15 species) followed by cummin (10 genera and 15 species), fennel (9 genera and 12 species), anise (8 genera and 11 species) and coriander seeds (7 genera and 10 species).

Genera and species	Anise		Caraway		Coriander		Cummin		Fennel	
	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI
Total count	8700		5020		5340		7370		7320	
Aspergillus fumigatus Fresenius	7522	39	3862	40	4770	39	5362	40	4970	40
Emericella nidulans (Eidam) Vuill.	228	22	290	30	58	12	764	32	880	23
Aspergillus terreus Thom	168	20	130	20	22	6	116	12	58	9
Emericella nidulans var. *latus* Thom & Raper	46	5	144	12	10	3	272	10	434	6
Emericella quadrilineata (Thom & Raper) Benjamin	34	3	50	6	2	1	84	3	214	4
Emericella rugulosa (Thom & Raper) Benjamin	12	1	30	4	-	-	2	1	163	3
Aspergillus fumigatus var. *albus* Rai, Twari & Agrawal	2	1	-	-	-	-	-	-	-	-
Aspergillus niger Van Tieghem	-	-	2	1	2	1	[illegible]	2	-	-
Rhizomucor pusillus (Lindt) Schipper	382	26	120	17	350	22	148	20	164	19
Mucor sp.	190	14	104	12	108	11	80	18	332	13
Malbranchea pulchella var. *sulfurea* (Miehe) Cooney & Emerson	26	6	24	7	4	1	20	5	12	5
Paecilomyces variotii Bainier	6	2	42	10	6	2	14	3	18	5
Humicola grisea var. *thermoidea* Cooney & Emerson	8	4	22	7	-	-	4	2	4	2
Humicola hyalothermophila Moubasher, Mazen & Abdel-Hafez	-	-	-	-	-	-	2	1	-	-
Chaetomium thermophilum var. *coprophilum* Cooney & Emerson	4	1	28	3	-	-	14	2	-	-
Sepedonium chrysospermum (Bull.:Fr.) Link	-	-	4	2	2	1	2	1	4	1
Myceliophtora thermophila (Apinis) Oorschot	-	-	8	2	-	-	-	-	-	-
Melanocarpus albomyces (Cooney [illegible] Emerson) V.Arx	-	-	2	1	-	-	-	-	-	-
Talaromyces dupontii Griffon & Maublanc	-	-	-	-	-	-	2	1	-	-
Thermomyces lanuginosus Tsikinsky	-	-	-	-	-	-	-	-	2	1
Sterile mycelium	68	19	152	19	6	2	118	15	64	9

Table 1 — Numbers of cases of isolation, out of 40 samples (NCI), of fungal genera and species and their total counts (TC) calculated per g dry seeds of each of anise, caraway, coriander, cummin and fennel in every sample tested on glucose — Czapek's agar at 45°C. High occurrence, between 20-40 cases ; moderate occurrence, from 10-19 cases ; low occurrence, from 5-9 cases ; rare occurence, less than 5 cases.

Tableau 1 — Genres et espèces de champignons isolés à partir de 5 types de graines (anis, carvi, coriandre, cumin, fenouil) sur milieu czapek (glucose) à 45°C.

Aspergillus fumigatus and *E. nidulans* were the most prevalent fungi. They emerged in 97.5% and 55%, 100% and 75%, 97.5% and 30%, 100% and 80%, and 100% and 57.5% of the samples contributing 86.5% and 2.6%, 76.9% and 5.8%, 89.3% and 1.1%, 72.8% and 10.4%, and 67.9% and 12% of total fungi, respectively. Reports from Malaysia showed that *A. fumigatus* was also the most frequent species on freshly harvested rice seeds (KUTHUBUTHEEN, 1979). *A. terreus* recovered in high frequency of occurrence (50% of the samples) from anise and caraway seeds, retreated to a backward situation in the other three seed types (15%, 30% and 22.2% of the samples). *E. nidulans* var. *latus* also isolated in moderate frequency of occurrence from caraway (30% of the samples) and cummin (25%) seeds, was of low or rare frequency in the other seed types (12.5%, 7.5% and 15% of the samples). The preceding species were also observed, but with variable densities and frequencies, from Egyptian peanuts, bean, lupine and pea seeds (ABDEL-HAFEZ & SHOREIT, 1986 ; MOUBASHER & al., 1979), as well as from Nigerian palm produce (OGUNDERO, 1981a). *E. quadrilineata*, *A. fumigatus* var. *albus*, *E. rugulosa* and *A. niger* were less common on all seeds.

Rhizomucor pusillus was also common on most types of seeds comprising 65%, 42.5%, 55%, 50%, and 47.5% of the samples, respectively. This species was previously encountered, but with variable densities and frequencies, from Egyptian soils, some types of seeds and self-heating materials (MOUBASHER & al., 1979, 1982), as well as from freshly harvested rice seeds from Malaysia (KUTHUBUTHEEN, 1979).

Paecilomyces variotii, moderately encountered from caraway seeds (25.0% of the samples), was less frequent on the remainder types of seeds (5-12.5%). MOUBASHER & al. (1979) isolated *P. variotii* in rare frequency of occurence (5% of the samples) from peanut seeds under the same experimental conditions.

Truly thermophiles such as *Malbranchea pulchella* var. *sulfurea*, *Humicola grisea* var. *thermoidea*, *H. hyalothermophila*, *Thermomyces lanuginosus*, *Chaetomium thermophilum* var. *coprophilum*, *Myceliophtora thermophila*, *Melanocarpus albomyces* and *Talaromyces dupontii* occured in reduced frequencies on one or several types of seeds tested (Table 1). MOUBASHER & al. (1982) found that the majority of these thermophiles were active colonizers of wheat and broad-bean straw composts. They also reported that wheat and broad-bean straws lost nearly half of their dry weight after 60 days of composting ; this loss was mainly attributed to the activity of thermophilic fungi. The remaining genera and species isolated (Table 1) were less frequent. Most of these fungi were reported from strored moist barley grains (MULINGE & APINIS, 1969), peanut (MOUBASHER & al., 1979), some leguminous seeds (ABDEL-HAFEZ & SHOREIT, 1986) and from different types of dates in Aswan area (NASSAR, 1986). *T. lanuginosus* was one of the most frequent species on freshly harvested rice seeds from Malaysia (KUTHUBUTHEEN, 1979), as well as from Nigerian palm produce (OGUNDERO, 1981a). FLANNIGAN & SELLARS (1972) found that *A. fumigatus*, *A. nidulans* (= *E. nidulans*), *A. terreus*, *M. pusillus* (= *Rh. pusillus*) and *T. lanuginosus* strains recovered from barley kernels were able to degrade arabinoxylan and carboxymethyl cellulose. They also reported (in 1977) that *A. fumigatus*, *M. pusillus* (= *Rh. pusillus*) and *T. lanuginosus* produced amylase, ß-glucosidase and ß-xylosidase. SELLARS & al. (1976) indicated that *A. fumigatus* could degrade barley husk and produce 1,4 ß-glucanase and ß-glucosidase when grown on eigher barley husk or crystalline cellulose. Thermophilic fungi were found to cause serious deterioration during storage of palm kernels (OSO, 1979), a considerable decrease in the oil contents of the palm fruit chaffs and the palm kernels by *C. thermophilum*, *T. lanuginosus* and *Torula ther-*

mophila (OGUNDERO, 1981b). Occurrence of these thermophilic or thermotolerant fungi in addition to mesophiles on the seeds of different plants which are grown in Egypt for their nutritive or medicinal values, calls for cautions handling during transport, storage and processing since numerous of these fungi were reported to be pathogenic to man and farm animals (COONEY & EMERSON, 1964 and LACEY, 1975).

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